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IMMUNE RESPONSES TO MHC HOMOZYGOUS LYMPHOID CELLS IN MURINE F1 HYBRID RECIPIENTS: IMPLICATIONS FOR TRANSFUSION ASSOCIATED GRAFT-VERSUS HOST DISEASE

BY

L.D. FAST, C.R. VALERI, AND J.P. CROWLEY

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

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ABSTRACT

Graft-versus-host disease(GVHD) is currently encountered following bone marrow transplantation and transfusion. GVHD associated with transfusion(TA-GVHD) in apparently immunocompetent recipients has been recently reported with increasing frequency. A consistent finding in many of these cases is that the recipient received blood from a donor homozygous for one of the the recipient's HLA haplotypes. However the observed frequency of TA-GVHD is much lower than the estimated probability of this donor/recipient combination. The potential role of recipient immune responses in controlling TA-GVHD was investigated using an analogous murine model where GVHD is induced by the injection of parental lymphoid cells into unirradiated F_1 hybrid recipients. The effect of various immune manipulations of the recipient on GVHD induction was assessed by determining the number of donor lymphoid cells required to induce GVHD responses. While depletion of recipient $CD4^+$ cells increased the number of donor cells needed to induce GVHD, depletion of recipient $CD8^+$ and natural killer cells resulted in fewer donor cells being needed to induce a GVHD response . These studies suggest a central role for functioning recipient CD8 and natural killer(NK) cells in the downregulation of TA-GVHD development in recipients who receive blood from a haploidentical donor.

INTRODUCTION

Graft-versus-host disease(GVHD) can occur following bone marrow transplantation(BMT), solid organ transplantation or transfusion of leukocyte containing blood.[1-3] The GVHD that follows BMT is clinically similar to the GVHD that is associated with blood transfusion(TA-GVHD) except that the TA-GVHD is almost invariably fatal within 30 days, while BMT-GVHD can often be controlled. Possible factors that may explain the greater intensity of the TA-GVHD response include the immune status of the recipient, the number of lymphoid cells transfused and the viability of the lymphocytes which depends on the length of time that the blood had been stored. Particular emphasis has been placed on the seeming lack of recipient immune response in the development of TA-GVHD.[4] All of the early reports of TA-GVHD were in patients who expressed impaired immunity as a result of inherited immunodeficiency, or as a result of receiving immunosuppressive or chemotherapeutic drugs. However recent studies have found that TA-GVHD could also occur in patients who were presumed to be immunocompetent. In many of the cases where both donor and recipient were HLA typed it was found that the HLA heterozygous recipient had received blood from a donor that was homozygous for one of the recipient's HLA haplotypes.[5-8] In this situation, the recipient lymphocytes would perceive the donor cells as self and thus would not respond to donor cells while the donor cells would recognize the recipient cells as foreign and generate a response. Thus it would be predicted that the only immune response possible in this situation would be a donor anti-recipient response. These findings would indicate that a lack of recipient anti-donor response is a critical element in the development of TAGVHD and may contribute to the severity of TA-GVHD. However, if this is true why have there been no reported TA-GVHD cases in severely immunodeficient AIDS patients? Moreover, theoretical calculations of the frequency of a HLA heterozygous recipient receiving blood from a homozygous donor would indicate that the incidence of

TA-GVHD in immunocompetent individuals is much lower than its predicted frequency implying that a protective host response to donor lymphocytes must exist.[8, 9]

Determination of an explanation for these observations is difficult to study in human patients because of 1) the lack of routine HLA typing for donor and recipients, 2) the variables introduced by the underlying conditions which require a blood transfusion and 3) the limited ability to manipulate the immune system. A mouse model of GVHD enables the investigator to overcome many of these shortcomings with the expectation that the findings would be applicable to the human patients because of the many similarities of the murine and human immune system. A mouse model of GVHD that mimics the immune conditions in TA-GVHD has been extensively studied in this laboratory.[10-14] GVHD is induced by the intravenous injection of homozygous parental spleen cells into unirradiated immunocompetent F_1 hybrid recipients. This is analogous to the HLA combination in humans which appears to be conducive to the development of TAGVHD. The resulting murine GVHD is similar to TA-GVHD because it induces severe hypoplasia of the lympho-hemopoietic system leading to severe pancytopenia.[15] This murine model was used to assess the role that the recipient immune system plays in the regulating GVHD reponses in this donor/recipient combination and the results indicated that functional recipient $CD8^+$ and NK cells play an important role in preventing GVHD in this donor/recipient combination.

MATERIALS AND METHODS

Mice. C57BL/6, CBA and B6D2F$_1$ mice were obtained from Jackson Laboratory, Bar Harbor, Maine. All protocols using mice have been approved by the Rhode Island Hospital Animal Welfare Committee.

Antibodies. The antibodies used in these experiments include 2.43(rat IgG2b, anti-Ly 2.2, ATCC)[16], GK1.5(rat IgG2b, anti-L3T4, ATCC)[17] and YTH 89.1.8(rat IgG2b, anti-human glycophorin, obtained from Dr. Herman Waldmann).[18] Commercially obtained antibodies included rabbit anti-asialo GM1(986-1001, Wako, Richmond, VA) which is used for in vivo depletion of NK cells.[19, 20]

In vivo administration of lymphoid cells and other reagents. Lymphoid cells were injected intravenously in the lateral tail vein of recipient mice by injecting the indicated number of donor cells in 0.2 ml PBS. Spleen cells were obtained from the recipient mice on day 14 following injection of donor cells for functional testing unless indicated otherwise. Some of the recipients had also been injected i.p. with 1 ml of monoclonal antibody culture supernatant 5 days or 100 ul of anti-asialo GM1(diluted 1:5 with PBS) 1 day prior to use as recipients. Some of the recipients were injected i.p. with 200 ug of poly I:C(P-5764, Sigma, St. Louis, MO) in PBS 1 day prior to injection of donor cells. Poly I:C induces the production of interferon which activates NK cell activity.[21]

Functional assays. Spleen cells obtained from recipient B6D2F$_1$(H-2$^{b/d}$) mice previously injected with allogeneic C57BL/6(H-2^b) cells were tested for their functional capacity as previously described.[11] The ability of the spleen cells to lyse 10^4 ^{51}Cr-labelled P815(H-2^d) target cells at the effector to target ratio of 150:1, 75:1, 37.5:1, and 19:1 was measured in a 4 hour lysis assay. Cytokine production by the spleen cells from

GVHD mice was measured by culturing 1.25×10^6 cells/ml of MLC medium alone or by including Con A(5ug/ml, C-2010, Sigma) or LPS(10 ug/ml, 3923-25, Difco, Detroit, MI) to stimulate cytokine production. Con A stimulates the production of interferon-γ(IFN-γ) and LPS stimulates the production of tumor necrosis factor-α(TNF-α).[22] The supernatants were collected and stored frozen at -20°C until until levels of IFN-γ and TNF-α were measured using ELISA assays(80-2802-00 and 1557-00, Genzyme, Cambridge, MA).

RESULTS

Injection of C57BL/6 spleen cells into B6D2F$_1$ recipients results in an acute form of GVHD. One of the hallmarks of acute GVHD is the presence of CD8$^+$ cytolytic T lymphocytes of donor origin which specifically lyse target cells expressing recipient MHC antigens.[11] In these experiments the effect of manipulating the recipient was evaluated by determining the number of C57BL/6 splenic donor cells required to generate detectable donor anti-recipient CTL as a measure of acute GVHD. The first set of experiments compared the effects of depleting recipient CD4$^+$ or CD8$^+$ cells on the development of GVHD. The results indicate that depletion of recipient CD8$^+$ cells permitted 4 fold fewer donor cells to generate an acute GVHD response(Figure 1). In contrast, depletion of CD4$^+$ cells resulted in additional donor cells being required to induce an acute GVHD response. NK cells have also been implicated in removal of allogeneic cells.[23] Depletion of NK cells was accomplished by administration of anti-asialo GM1 antisera and was found to result in a 2 fold reduction of the number of donor cells required to induce GVHD(Figure 2). Consistent with this finding, the presence of increased NK cell activity as a result of poly I:C injection required at least 2 fold more donor cells to induce a GVHD response(Figure 3).

One possible explanation for the minimum number of donor cells that are required to induce an acute GVHD response is that a certain number of donor cells are lost as a result of nonspecific trapping. If this were the case addition of carrier cells syngeneic with the recipient should permit fewer number of C57BL/6 donor cells to induce a GVHD response. Addition of 50 x 10^6 B6D2F$_1$ cells to varying numbers (10 -100 x 10^6) of C57BL/6 donor cells did not effect the GVHD responses of the C57BL/6 cells(data not shown). Induction of TA-GVHD is often associated with the receipt of a blood transfusion from a donor homozygous for one the the recipient's MHC haplotypes. One possibility is that the appearance of TA-GVHD in this situation could be facilitated by the immune responses that result from a blood transfusion received simultaneously from a donor that is allogeneic to both the recipient and the homozygous donor. This possibility was assessed by adding from 10 - 75 x 10^6 allogeneic CBA/J(H-2^k) spleen cells to varying numbers of C57BL/6(H-2^b) donor spleen cells and measuring the GVHD response that occurred when these cells were injected into B6D2F$_1$ (H-$2^{b/d}$) recipients. The results of seven experiments indicated only minimal and inconsistent changes were detected in the ability of the C57BL/6 cells to induce a GVHD response in this model system as a result of coinjecting allogeneic donor cells(data not shown).

Because production of cytokines such as IFN-γ and TNF have been implicated in the pathogenesis of GVHD[24], the production of TNF and IFN-γ was compared to the development of donor anti-recipient CTL following the injection of varying numbers of donor lymphoid cells into unirradiated B6D2F$_1$ recipients. TNF-α production and CTL generation were found to be inversely correlated in that there was no TNF-α production in those combinations which generated donor anti-recipient CTL(Figure 4). In contrast, increased IFN-γ production correlated with increased CTL activity and could still be detected at a donor cell dose which did not induce donor anti-recipient CTL (25 x 10^6 cells, Figure 4).

DICUSSION

TA-GVHD and the acute form of BMT-GVHD manifest similar symptoms except for the more severe pancytopenia that is found in TA-GVHD. Why is TA-GVHD so much more severe with death occuring in almost all cases within 30 days? A possible explanation is that the conditions that permit TA-GVHD generate a much stronger donor anti-recipient response. This could be due in part to a lack of recipient anti-donor responses. Although BMT recipients undergo intensive conditioning prior to receiving the bone marrow inoculum, there are recipient anti-donor responses which can still function. This has been shown by the finding of an increased incidence of graft rejection following transplant of T cell-depleted bone marrow cells.[25] In this regard it is interesting that in most of the cases of TA-GVHD that have been analyzed so far the the HLA heterozygous recipient had received blood from a donor that is homozygous for one of the recipient's HLA haplotypes. In this combination a lack of a specific recipient anti-donor response would be predicted. This is because T lymphocytes which undergo a negative selection step have been selected not to respond to self MHC molecules.[26] In this combination T cells would see leukocytes from the donor as self while the donor cells recognize the allogeneic HLA antigens expressed on recipient cells as foreign. Although this combination would be predicted to limit the response to a donor anti-recipient response it is clear that the frequency of TA-GVHD is much lower than the calculated frequency of which a HLA heterozygous recipient receives blood from a donor homozygous for one the recipient's HLA haplotypes.[9]

What is the explantion for the discrepancy between the frequency of the donor/recipient combination and the incidence of TA-GVHD? One possible explanation is that although the recipient lymphocytes are unable to mediate specific immune responses in this donor/recipient combination they may generate nonspecific responses which are able to regulate the donor cells in this situation. In this study we have shown that recipient $CD8^+$

cells and NK cells are capable preventing GVHD responses of donor cells. How can these cells nonspecifically mediate this effect? Activation of recipient NK cells has been shown to promote the elimination of injected allogeneic cells.[23] Cytokines such as IFN-γ which are produced as a result of the donor anti-recipient response could activate recipient NK cells and enhance their abilty to eliminate the donor cells. Nonspecific functions mediated by $CD8^+$ cells include veto cell activity. Veto cells are cells which inactivate CTL precursor cells which recognize and bind to the veto cell and have been implicated in the development of transplantation tolerance.[27] The specificity is supplied by the CTL precursor cells not the veto cells. In the same murine GVHD model used for these experiments recipient F_1 $CD8^+$ cells have been shown to inhibit the donor cytolytic response by acting as veto cells.[13, 14] These B6D2F_1 veto cells were found to be radiosensitive, inhibited by anti-CD8 and potentiated by incubation with IL-2. Similarly, $CD8^+$ cells which inhibited donor anti-host responses could be identified in post transfusion responses.[28] These findings also provide an explanation for the lack of reported TA-GVHD in AIDS patients following transfusion. Although these patients are immunosuppressed because of a lack of $CD4^+$ cell function, $CD8^+$ cells are still functional in these patients until the advanced stages of the disease.[29]

These results would suggest that procedures or conditions which result in the lack of recipient CD8 and NK cell function would be conducive to the development of TA-GVHD. For example anesthesia and surgery has been shown to be associated with a depression in cell-mediated immunity.[30] This is manifested by a decrease in T and NK cell number and by depressed in vitro responses to mitogens and antigens and by depressed DTH responses in vivo. The magnitude of the depression in immune responses is increased as the extent of the surgery increases. The development of TA-GVHD has often been found to be associated with the recipient receiving blood when undergoing major surgery correlating the loss of recipient immune function with increased incidence of TAGVHD.[5, 6]

It has been proposed that the symptoms associated with GVHD are the result of dysregulation of the production of inflammatory cytokines including IL-1, IL-2, and TNF-α.[24] This hypothesis has been supported by the finding that administration of TNF-α generates GVHD lesions and inhibition of TNF reduces the severity of GVHD.[24] Inhibition of IL-1 using IL-1 receptor antagonist also blocked the development of GVHD symptoms.[31, 32] These studies have been done in murine models of bone marrow transplantation using irradiated recipients. Analysis of the production of cytokine mRNA 14 days after the injection of C57BL/6 spleen cells into unirradiated $B6D2F_1$ recipients detected increased mRNA levels for IL-1α, IL-10, IFN-γ, and MIP-1α and decreased mRNA levels for TNF-β.[33] Unchanged mRNA levels for TNF-α were detected. Thus our findings of increased IFN-γ correlated with increased levels of mRNA. However despite no change in mRNA levels there was decreased production of TNF-α. Additional studies using mice which are selectively unable to produce cytokines such as IFN-γ or TNF-α or mediate cytolytic activity will help distinguish whether cytokine production or cytolytic activity is important for the development of GVHD.

These findings would suggest that any recipient with impaired CD8 and/or NK cells or who will be undergoing a procedure which inhibits the function of CD8 and/or NK cells would be at increased risk for developing TAGVHD. This would be especially true if they also received a blood transfusion from an individual that is homozygous for one of their HLA haplotypes and if the blood contains large numbers of viable lymphocytes. Since the threshold number of lymphocytes in blood products below which TA-GVHD would not occur is unknown, irradiation of transfused blood in the clinical settings where impaired recipient NK or $CD8^+$ cell function has been identified would be expected to reduce the risk of developing TA-GVHD.

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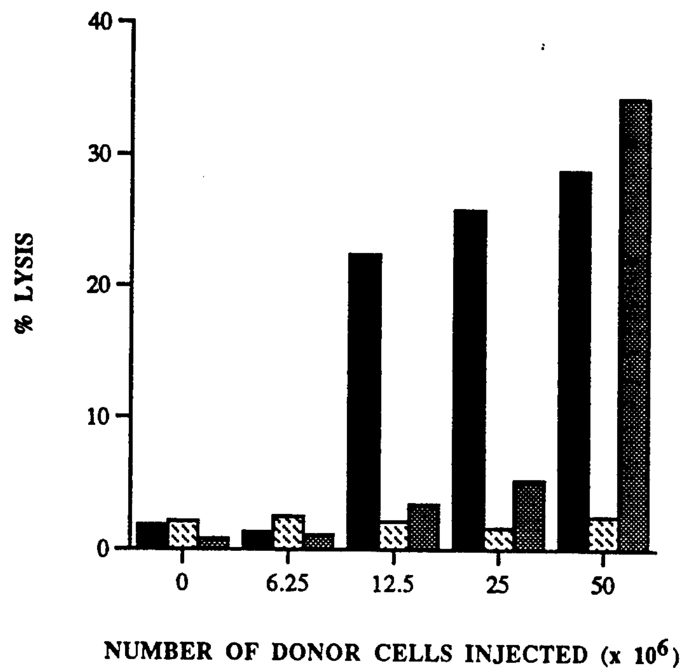
Figure 1. The effect of T cell subset depletion on the number of donor splenocytes required to induce a GVHD response. $B6D2F_1$ recipient mice were injected with anti-CD4(GK 1.5,), anti-CD8(2.43,), or control antibody(YTH 89.1.8,) and then were injected with the indicated number of C57BL/6 donor spleen cells. The presence of donor anti-recipient CTL was assessed by testing the ability of spleen cells obtained from the recipient mice 14 days after injection to lyse P815 target cells(150:1 effector to target ratio). This data is from a representative experiment of 3 such experiments.

Figure 2. The effect of depleting NK cells on the number of donor splenocytes required to induce a GVHD response. $B6D2F_1$ mice were injected with rabbit anti-asialo GM1 ( ) or normal rabbit serum( ) and then injected with the indicated number of C57BL/6 spleen cells. The presence of donor anti-recipient CTL was assessed by testing the ability of spleen cells obtained from the recipient mice 14 days later.to lyse P815 target cells(150:1 effector to target ratio). This is a representative experiment of 3 such experiments.

Figure 3. The effect of injecting poly I:C on the number of donor splenocytes required to induce a GVHD response. $B6D2F_1$ mice were injected with poly I:C( ) or PBS( ) and then injected with indicated number of C57BL/6 donor spleen cells. The presence of donor anti-recipient CTL was assessed by testing the ability of spleen cells obtained from the recipient mice 14 days later.to lyse P815 target cells(150:1 effector to target ratio). This is a representative experiment of 3 such experiments.

Figure 4. The effect of donor cell dose on donor anti-recipient CTL responses and the production of TNF-α and IFN-γ. B6D2F$_1$ mice were injected with the indicated dose of C57BL/6 spleen cells. Fourteen days later the spleen cells were obtained from these recipient mice and the presence of donor anti-recipient CTL was assessed by testing the ability of spleen cells obtained from the recipient mice 14 days later.to lyse P815 target cells(150:1 effector to target ratio, C), for the cells ability to produce TNF-α(A) when cultured overnight in medium(■) or with LPS(▨), or for IFN-γ production(B) in overnight culture with medium(■) or with Con A(▨). This is a representative experiment of 3 such experiments.



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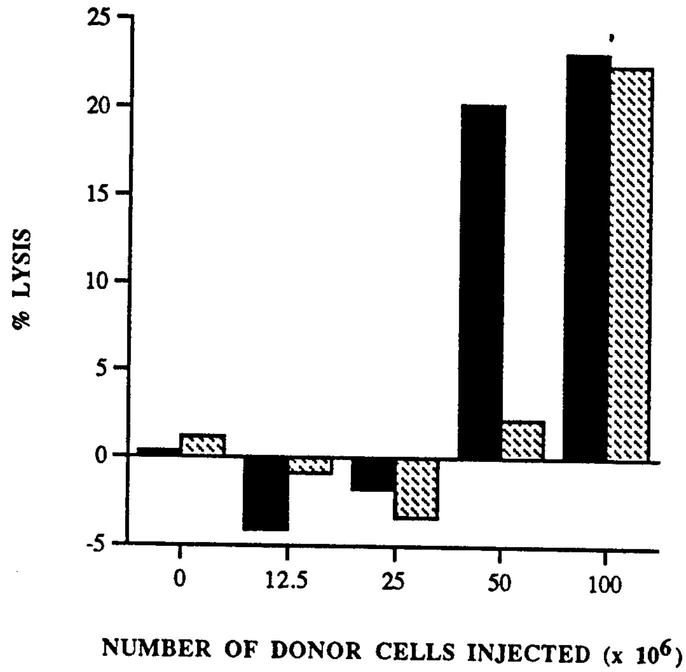
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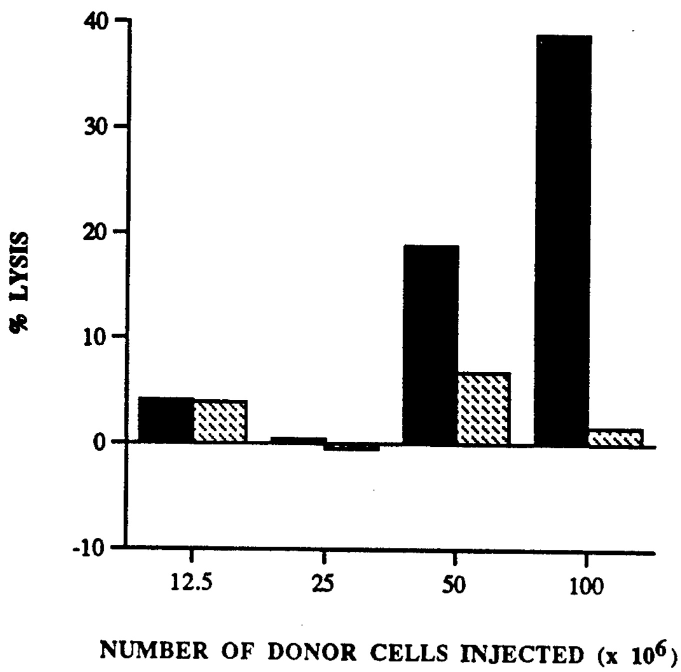

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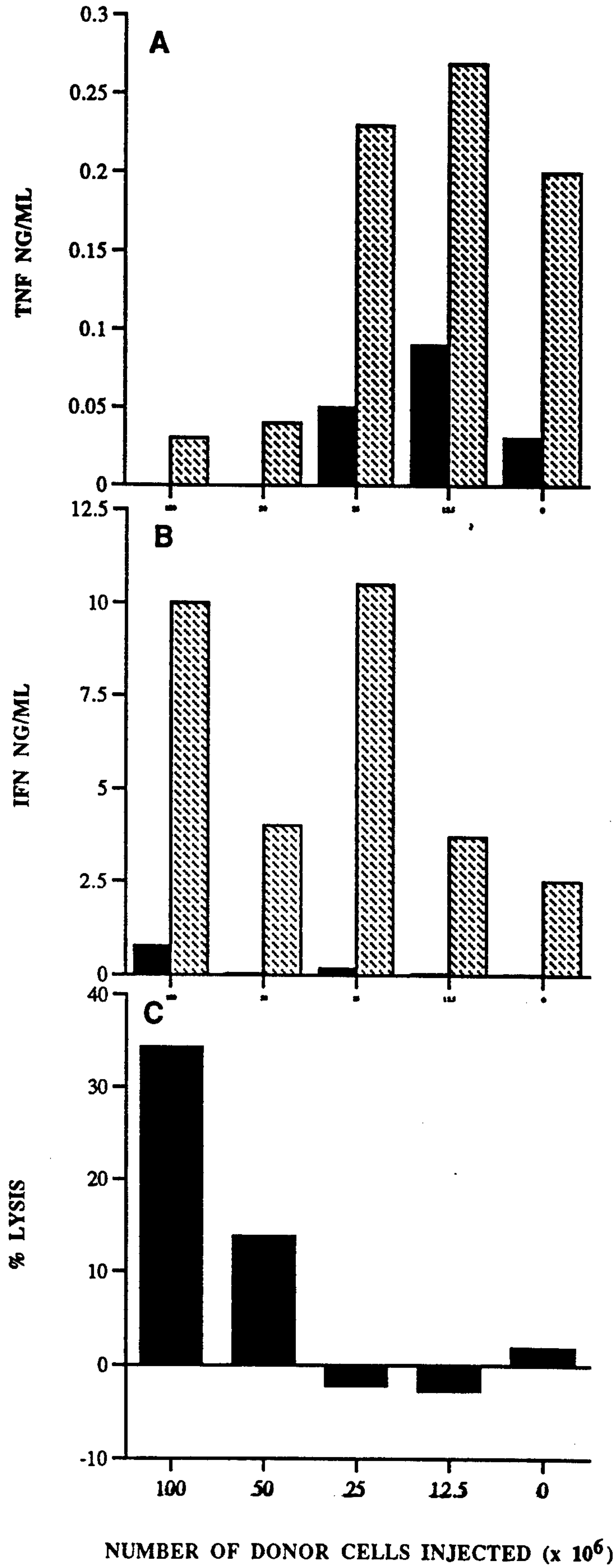
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